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Distribution of CCR5 genotypes and HLA Class I B alleles in HIV-1 infected and uninfected injecting drug users from Rio de Janeiro, Brazil

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ABSTRACT

Host genetic factors play an important role in the HIV epidemic dynamics, and have been considered in studies assessing susceptibility/resistance to HIV-1 infection as well as clinical evolution. Class I and Class II HLA alleles have been associated with the heterogeneity of HIV-1 infection susceptibility, as protective or risk factors for HIV-1 transmission. Moreover, a 32-base pair deletion in the HIV-1 CCR5 gene-coding region confers resistance to HIV-1 infection in homozygous individuals for the deleted allele.

In this study, DNA samples from HIV-1 infected and uninfected injecting drug users (IDUs) from Rio de Janeiro were PCR amplified to determine CCR5 genotypes based on the presence of the CCR5Δ32 mutation and typed for the HLA-B locus, in an attempt to assess possible associations between these genetic factors and susceptibility/resistance to HIV-1 infection.

The distribution of CCR5 genotypes between the two IDU groups did not differ. The homozygous mutant genotype Δ32/Δ32 was not found in this study. Except for HLA-B*45 (4.0% vs. 3.0%; $p = 0.04$) and for B*51 (12.1% vs. 4.4%; $p = 0.002$), no statistically significant differences were made evident when analyzing the frequencies of each HLA-B allele between Caucasian and non-Caucasian IDUs. The most frequent HLA-B alleles were B*15; B*35; B*44 and B*51. Although some differences in the allele frequencies could be observed between the two IDU groups, none of these was statistically significant. Therefore, no putative association between these genetic markers and susceptibility/resistance to HIV-1 infection could be made evident in the present study. So far, the assessment of genetic markers among the IDU population has been restricted to North American, European, and Asian studies and this report represents a pioneer descriptive study of the distribution of CCR5 genotypes and HLA-B alleles in Rio de Janeiro, Brazil.

1. Introduction

Several studies have been investigating the influence of host genetic factors in the dynamics of HIV epidemic. Together with viral characteristics, infectious cofactors, individual behaviors and environment factors, host genetics can be considered an important agent affecting HIV-1 susceptibility and disease progression (Royce et al., 1997; MacDonald et al., 2000). The considerable heterogeneity in the AIDS epidemic is at least partially determined by genetic variations associated with virus replication and immunity (O'Brien & Nelson, 2004). In addition to the two major families of host's genes—chemokine receptor CCR5 and Major Histocompatibility Complex (MHC) shown to be associated with HIV transmission and disease progression (reviewed by Theodorou et al., 2003), other polymorphisms related to CCR2, CX3CR1, SDF-1, MIP-1A, MIP-1B, RANTES, IL-10 and IL-4 have already been described (reviewed by Telenti, 2005).

In some ethnic groups (e.g. Caucasians), a naturally occurring mutation was detected in the CCR5 co-receptor, called CCR5Δ32. It consists of a 32-base pair deletion in the HIV-1 CCR5 gene coding region, leading to the production of a truncated protein that is not transported to the cell surface, and, therefore, confers resistance to HIV-1 infection in individuals homozygous for the deleted allele and partial protection against HIV-1 for disease progression in heterozygotes (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996; Huang et al., 1996; Marmor et al., 2001; reviewed by Sullivan et al., 2001).

The MHC contains the most polymorphic loci in humans, the HLA Class I and Class II genes – whose products are fundamental to

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acquired immune responses – constituting important host genetic risk factors for infectious diseases, with associations over a hundred different diseases (reviewed by Trachtenberg & Erlich, 2001; Carrington & O'Brien, 2003). Due to the extensive polymorphism of HLA genes in the population, the immune response against a pathogen tends to vary among individuals.

Several Class I and Class II HLA alleles have been associated with the heterogeneity of HIV-1 infection susceptibility, as protective or risk factors for HIV-1 transmission. HLA Class I B35 allele has been associated with resistance to infection in a cohort of HIV-exposed but uninfected Gambian sex-workers (Rowland-Jones et al., 1995). A protective association in a group of African sex-workers from Nairobi include the HLA-A2-A*6802 supertype, while a susceptible association was found for HLA-A*2301 (MacDonald et al., 2000). Protection against HIV-1 infection was associated with the presence of alleles B*44 and B*55 in HIV-1 infected patients in Argentina, whereas B*18 and B*39 had a suggested role in susceptibility to HIV-1 infection (de Sorrentino et al., 2000). Among HIV-1 positive Italian patients, a higher frequency of HLA-A2 has been reported, while HLA-B52 was decreased, acting as risk and protective factors, respectively (Fabio et al., 1990). Moreover, alleles such as HLA-B*57, HLA-B*27 and HLA-B*51 are associated with successful control of HIV infection and disease progression (Goulder & Watkins, 2008).

Recently, Kiepiela et al. (2004) investigated the contribution of different HLA Class I molecules within the CD8+ T-cell activity directed against HIV, and reported that a substantially greater selection pressure is imposed on HIV-1 by HLA-B alleles than by HLA-A, indicating that the principal focus of HIV-specific activity is at the HLA-B locus. This evidence reinforces the relevance of the study and characterization of the distribution of these alleles, since HLA-B gene frequencies in the population are those likely to be most influenced by HIV disease (Kiepiela et al., 2004).

In previous papers we analyzed a population of 608 IDUs from Rio de Janeiro, Brazil, assessing socio-demographic and behavioral data, as well as estimating incidence and seroprevalence of HIV-1 infection in such population (Teixeira et al., 2004; Hacker et al., 2005). In the present study we extend these analyses assessing the distribution of CCR5 genotypes (with focus on the investigation of Δ32 mutation occurrence) and the frequency of the different HLA Class I B alleles in an attempt to assess possible associations between these genetic factors and susceptibility/resistance to HIV-1 infection in the IDU population.

2. Material and methods

2.1. Study group

Injecting drug users (including both active and former IDUs) from Rio de Janeiro were recruited from September 1999 to December 2001, as part of a multicenter study (WHO Multicenter Study Phase II). After signing an informed consent form, the individuals were interviewed using a standard questionnaire addressing socio-demographic data, sexual and injecting risk behaviors, and information on the ethnic background and health status of interviewees (Hacker et al., 2005). Information about blood samples collection and storage, as well as determination of HIV serostatus, CD4 counts, viral load and DNA extraction were described elsewhere (Teixeira et al., 2004).

2.2. CCR5 genotyping

DNA samples from 48 HIV-1 seropositive and 558 HIV-1 seronegative IDUs were PCR amplified in order to determine CCR5 genotypes based on the presence of the CCR5Δ32 mutation. Primers were used to amplify a 182 bp fragment of the wild-type allele and a 150 bp fragment of the mutant allele, according to the PCR protocol described by Grimaldi et al. (2002). The amplified fragment corresponded to positions 547–729 of the human CCR5 gene (GenBank accession number: U95626), covering the Δ32 mutation region. Cycling conditions were: 1 cycle 94 °C 5 min; 30 cycles 94 °C 1 min, 60 °C 30 s, 72 °C 2 min; 1 cycle 72 °C 10 min. The PCR amplified products (10 μl) were submitted to electrophoresis on 12% polyacrilamide gel (200 V; 3 h 30′) and visualized by UV trans-illumination. The CCR5/CCR5 wild-type genotype was detected by a single band of 182 bp while the heterozygote genotype CCR5/Δ32 was detected by 182 and 150 bp bands; and the homozygous mutant genotype Δ32/Δ32 by a single band of 150 bp.

2.3. HLA Class I B alleles typing

In order to type HLA-B alleles at allele group level, we used a combination of two commercial kits (INNO-LiPA HLA-B Multiplex Plus and INNO-LiPA HLA-B Update Plus, INNOGENETICS, Ghent, Belgium). The first one is intended for the nucleic acid amplification of the second to the fourth exon of HLA-B locus. DNA samples from 48 HIV-1 seropositive and 207 HIV-1 seronegative IDUs were PCR amplified according to the manufacturer's protocol. After this step, using the second kit, the PCR products were submitted to a hybridization assay, based on the line probe assay methodology. The identification of HLA-B alleles was performed by means of the LiRAS interpretation software for LiPA HLA v5.00 (INNOGENETICS, Ghent, Belgium).

2.4. Statistical analyses

HLA-B allele frequencies and genotypes were estimated by using the computer package PyPop (Lancaster et al., 2003). Deviations from Hardy–Weinberg equilibrium were calculated by using the method of Guo & Thompson (1992), also performed using PyPop. The frequencies of each HLA-B allele were compared between HIV-1-positive individuals and control subjects, as well as between the IDU population here studied and the Brazilian population data available at MHC database (see below), and p-values were calculated using Chi-square tests (or Fisher's exact tests, when appropriate), by means of the statistics program Epi Info Version 6 (Dean et al., 1995). Putative differences in the distribution of CCR5 genotypes between the two IDU groups were assessed in the same way.

The Brazilian population HLA-B allele frequency data were collected from the Major Histocompatibility Complex Database—dbMHC (Kitts et al., 2003), using the integrated resource anthropology/allele frequencies of the International Histocompatibility Working Group (IHWG) Projects available at this database (http://www.ncbi.nlm.nih.gov/mhc).

3. Results

The IDU population included in this study had a mean age of 37.1 (+/−9.9) years old and was composed by 47.1% of Caucasians, 35.7% of Mestizos (Biracial), and 17.2% of Black individuals. The HIV-1 positive IDU group included 37.5% of Caucasians, 37.5% of Mestizos, and 25.0% of Black individuals, whereas in the IDU HIV-1 negative group 49.3% were Caucasians, 35.3% Mestizos and 15.4% Black individuals. Although the proportion of Mestizos and Black individuals was higher in the HIV-1-positive group compared to the negative one, no statistically significant difference was found ($p = 0.771$ and $p = 0.115$, respectively). Gender differences ($p = 0.010$) were, however, observed in the proportion in the HIV-1 positive (81.3%) and HIV-1 negative (93.7%) IDU groups. Overall, 91.0% of the individuals included in the IDU group were

males, 64.3% were single, and 43% with 1–8 years of education (Hacker et al., 2005). HIV-HCV co-infection was detected in 16.8% of the IDUs included in this study (Oliveira et al., 2009).

In relation to the CCR5 genotyping, almost all (46 in 48) HIV-1 seropositive IDUs were homozygous (95.8%) for the wild-type allele (CCR5/CCR5 genotype), with only 2 individuals with one mutant allele (4.2%), which characterizes the heterozygote genotype CCR5/Δ32. Frequency of the Δ32 allele in this group was 0.021. In the control group, 522 individuals (93.6%) have the CCR5/CCR5 genotype and 36 (6.4%) showed the CCR5/Δ32 genotype. Frequency of the Δ32 allele in this group was 0.032. The homozygous mutant genotype Δ32/Δ32 was not found in this study. The distribution of CCR5 genotypes between the two IDU groups did not differ ($p = 0.75$), precluding any further assessment of the putative association between specific CCR5 genetic profiles and susceptibility to HIV-1 infection. We analyzed the CCR5 genotypes distribution considering the ethnic background of the IDUs, but no statistically significant association was detected.

Due to the miscegenation of the Brazilian population, we first analyzed the frequencies of each HLA-B allele between Caucasian and non-Caucasian (Mestizos and Black individuals) IDUs (data not shown). Except for HLA-B*45 (4.0% vs. 3.0%; $p = 0.04$) and for B*51 (12.1% vs. 4.4%; $p = 0.002$), no statistically significant differences were made evident. In consequence, the frequencies of HLA-B alleles are presented as a whole, irrespective of the ethnic background when comparing HIV-1 seropositive and HIV-1 seronegative IDU groups (Table 1). For the alleles HLA-B*45 and HLA-B*51 the frequencies for HIV seropositive and HIV seronegative groups were, respectively, evaluated for Caucasian and for non-Caucasian individuals, as follows: B*45 (0% HIV−, Caucasian vs. 2.8% HIV+, Caucasian [$p = 0.150$]; 3.3% HIV−, non-Caucasian vs. 1.7% HIV+, non-Caucasian [$p = 0.689$]); B*51 (12.7% HIV−, Caucasian vs. 8.3% HIV+, Caucasian [$p = 0.721$]; 4.3% HIV−, non-Caucasian vs. 5.0% HIV+, non-Caucasian [$p = 0.732$]).

Overall, deviation from Hardy–Weinberg equilibrium was not observed in the distribution of HLA-B genotypes in this IDU population (data not shown). In general, the most frequent HLA-B allele found in this study was B*15, with 17.7% of prevalence in HIV-1-positive group and 12.3% in the control group, followed by B*35 (7.3% and 9.9%); B*44 (9.4% and 8.5%, respectively) and B*51 (6.3% and 8.5%). Considering the results of the two IDU groups together ($N = 255$), independent of the ethnic background, these four more common alleles accounted for 39.3% of HLA-B overall frequency; another 14 middle frequent alleles with frequencies ranging from 2% to 6% accounted for 49.4% allele frequency and the remaining 12 alleles were considered rare, with frequencies lower than 2%, accounting for 11.3% allele frequency. Alleles B*13, B*38, B*49, B*54, B*56, B*78 and B*82 were absent in the HIV-1-positive IDU group, whereas no absence were observed in the HIV-1 negative control group. Although some differences in the allele frequencies could be observed between the two groups, none of these was statistically significant ($p > 0.05$).

The composition of the HLA-B genotypes of this IDU group was quite diverse, with few common genotypes among the 255 individuals studied, resulting from the combination of the four more common alleles cited above: 6 individuals presented the B*15:B*35 genotype; 3 presented the B*15:B*44 genotype; 4 presented the B*15:B*15 genotype and B*15:B*51 was found in 7 individuals. HLA-B heterozygous genotypes were found in the great majority of the individuals ($N = 236$), whereas the remaining nineteen individuals carry homozygous genotypes (four in the HIV-1 seropositive vs. fifteen in the seronegative IDU groups).

The frequencies of the HLA-B alleles in the IDU population (both IDU groups) and those reported for the Brazilian population (data deposited in the dbMHC), independent of the ethnic background, are described in the Table 2. It is important to point out that B*15 allele frequency is significantly increased ($p = 0.039$), while B*27 frequency is significantly decreased ($p = 0.032$) in the IDU population, compared to the Brazilian population data. The observed differences in the frequencies of the others HLA-B alleles were not statistically significant.

Table 1
Distribution of HLA-B alleles among 48 HIV-1 seropositive IDUs and 207 HIV-1 seronegative IDUs (control group) from Rio de Janeiro, Brazil.

Allele	HIV-1 seropositive $2n = 96$		HIV-1 seronegative (control group) $2n = 414$		*p-value*[a]	OR
	N	Allele frequency	*N*	Allele frequency		
B*07	7	0.073	21	0.051	0.390	1.47
B*08	2	0.021	24	0.053	0.196	0.35
B*13	0	0.000	5	0.019	0.590	0.00
B*14	4	0.042	21	0.055	1.000	0.83
B*15	17	0.177	44	0.123	0.054	1.81
B*18	2	0.021	13	0.039	0.748	0.66
B*27	2	0.021	5	0.012	0.619	1.77
B*35	7	0.073	33	0.099	0.823	0.91
B*37	2	0.021	2	0.005	0.158	4.47
B*38	0	0.000	11	0.031	0.230	0.00
B*39	3	0.031	14	0.043	1.000	0.92
B*40	3	0.031	9	0.022	0.706	1.45
B*41	3	0.031	4	0.014	0.122	3.37
B*42	2	0.021	12	0.029	1.000	0.71
B*44	9	0.094	33	0.085	0.652	1.19
B*48	1	0.010	2	0.007	0.460	2.21
B*49	0	0.000	12	0.027	0.135	0.00
B*50	3	0.031	6	0.019	0.378	2.24
B*52	2	0.021	4	0.009	0.308	2.22
B*53	2	0.021	13	0.031	1.000	0.67
B*54	0	0.000	1	0.002	1.000	0.00
B*55	2	0.021	1	0.002	0.089	8.96
B*56	0	0.000	2	0.007	1.000	0.00
B*57	6	0.063	12	0.036	0.119	2.28
B*58	6	0.063	18	0.050	0.419	1.50
B*78	0	0.000	2	0.005	1.000	0.00
B*81	3	0.031	7	0.017	0.404	1.91
B*82	0	0.000	1	0.002	1.000	0.00

N = observed number of a HLA-B allele; $2n$ = total number of alleles (allele count); OR = odds ratio.
[a] Chi-square test or Fisher's exact test.

4. Discussion

In the present study we report, for the first time, the distribution of CCR5 genotypes, as well as the distribution and frequencies of HLA-B alleles in HIV-1 infected and uninfected IDUs from Rio de Janeiro, Brazil.

The Brazilian population is genetically very diverse, with an elevated degree of miscegenation among Caucasians, Africans and Amerindians (reviewed by Alves et al., 2006) which might impact polymorphisms observed for several human genetic markers. Indeed, in an analysis of the distribution of HLA-B alleles in six diverse populations worldwide, the greatest HLA-B variation was detected within the Brazilian samples (Williams et al., 2001) and, as proposed by the authors, such considerable heterogeneity may reflect both ancient and recent mixing of ethnic groups.

Moreover, allele frequencies for HLA Class II alleles (Moraes et al., 1993; Vanderborght et al., 2007) and distribution of B*39 subtypes have been reported for subjects living in Rio de Janeiro (Moraes et al., 2004). Some studies have characterized the distribution of HLA Class I and Class II alleles in populations from other Brazilian regions (Goldberg et al., 1998; Louzada-Junior et al., 2001; Monte et al., 2004; Nigam et al., 2004; Ruiz et al., 2005). Despite the availability of HLA allele distribution, data reported by some Brazilian studies, knowledge about the HLA allelic profile in specific populations and their potential association with risk for

Table 2
Frequencies of HLA-B alleles of the IDU population from Rio de Janeiro, Brazil, and Brazilian population (data from the dbMHC[a]).

Allele	IDU n = 510 alleles		Brazilian (IHWG) n = 184 alleles		p-value[b]	OR
	N	Allele frequency	N	Allele frequency		
B*07	29	0.055	11	0.060	0.884	0.95
B*08	27	0.047	13	0.071	0.410	0.75
B*13	8	0.016	1	0.005	0.457	2.92
B*14	27	0.053	11	0.060	0.727	0.88
*B*15*	68	0.133	14	0.076	*0.039*	1.87
B*18	18	0.035	12	0.065	0.079	0.52
*B*27*	7	0.014	8	0.043	*0.032*	0.30
B*35	48	0.094	21	0.114	0.424	0.80
B*37	4	0.008	1	0.005	1.000	1.42
B*38	13	0.025	5	0.027	0.793	0.92
B*39	22	0.041	3	0.016	0.094	2.72
B*40	12	0.024	8	0.043	0.166	0.53
B*41	9	0.018	2	0.011	0.736	1.63
B*42	15	0.027	7	0.038	0.567	0.77
B*44	44	0.086	18	0.098	0.638	0.87
B*45	9	0.018	5	0.027	0.539	0.64
B*47	0	0.000	1	0.005	0.262	0.00
B*48	4	0.008	0	0.000	0.578	Undefined
B*49	12	0.022	4	0.022	1.000	1.08
B*50	11	0.022	6	0.033	0.410	0.65
B*51	42	0.080	15	0.082	0.972	1.01
B*52	6	0.012	2	0.011	1.000	1.07
B*53	15	0.029	3	0.016	0.428	1.80
B*54	1	0.002	0	0.000	1.000	Undefined
B*55	3	0.006	2	0.011	0.610	0.53
B*56	3	0.006	0	0.000	0.571	Undefined
B*57	21	0.041	4	0.022	0.237	1.90
B*58	27	0.053	5	0.027	0.153	2.00
B*67	0	0.000	1	0.005	0.262	0.00
B*78	2	0.004	0	0.000	1.000	Undefined
B*81	10	0.020	1	0.005	0.304	3.66
B*82	1	0.002	0	0.000	1.000	Undefined

N = observed number of a HLA-B allele; $2n$ = total number of alleles (allele count); OR = odds ratio.
[a] dbMHC, the Major Histocompatibility Complex Database. (http://www.ncbi.nlm.nih.gov/mhc).
[b] Chi-square test or Fisher's exact test.

disease acquisition is far from comprehensive. These data may vary considerably according to the studied population.

With respect to ethnic background, we verified that the IDU population included in this study, although recruited by a non-random sampling strategy, is roughly comparable to the general population of Rio de Janeiro, since the proportions of Caucasians, Mestizos, and Black individuals are similar to the stratification of the general population from Rio de Janeiro (IBGE - Demographic Census 2000. Available at: http://www.sidra.ibge.gov.br). With exception of HLA-B*51 and B*45 that were respectively more represented in Caucasian and non-Caucasian IDUs, all other alleles were equally distributed in this population. Ethnicity information retrieved from the IMGT/HLA Database for HLA-B*51 and HLA-B*45 confirms their association with Caucasian and Black populations, respectively (Hildebrand et al., 1992; Robinson et al., 2003).

A protective immunity against HIV infection represented by HIV-specific cytotoxic T-lymphocyte activity had already been reported in studies involving seronegative individuals who have been exposed to HIV without becoming infected (Rowland-Jones et al., 1993, 1995). Another study showed that heterogeneity in susceptibility to HIV-1 infection among highly exposed sex workers is associated with several HLA alleles (MacDonald et al., 2000), providing evidence that resistance to HIV-1 infection in the cohort studied is immunologically mediated. Moreover, associations of Class I and Class II HLA alleles and HIV-1 infection and progression to AIDS were reported by several studies and reviewed by some authors (Trachtenberg & Erlich, 2001; Carrington & O'Brien, 2003; O'Brien & Nelson, 2004). Taken together, these findings reinforce the essential role of HLA molecules in the differential susceptibility/resistance to HIV-1 infection.

In the present study, none homozygous mutant Δ32/Δ32 individual was found. This was not surprising, since the frequency of this genotype in the Caucasian population—the predominant ethnic background of the 38 heterozygote IDUs found here (who carry one mutant Δ32 allele)—is about 1% (Samson et al., 1996). Among healthy and ethnically admixed individuals from the south of Brazil, Δ32/Δ32 homozygotes had also not been detected (Vargas et al., 2006). With regards to HIV-1, individuals who are homozygous for CCR5Δ32 were recently considered as the only well-characterized human population escape mutants (Telenti, 2005). Allelic frequencies of the mutant Δ32 allele are higher in Caucasians (about 10%), and absent or very rare in Western and Central Africa, Japanese and Amerindian populations (Samson et al., 1996; Lucotte, 1997; Martinson et al., 1997). The mutant Δ32 allele frequencies reported here (0.021 for the HIV-1 seropositive IDU group; 0.032 for the HIV-1 seronegative IDU group; and 0.031 overall) are similar to the overall frequency (0.022) reported for three Brazilian population groups (Grimaldi et al., 2002). In this study, Grimaldi and co-workers reported for the first time a Brazilian Δ32 homozygous case among a German-descended group of blood donors from Southern Brazil. In an analysis of Spanish HIV-1 seropositive injecting drug users, the frequency of the Δ32 mutant allele was 0.040 (Alvarez et al., 1998).

In the present paper, some alleles were found to occur more frequently in one of the two IDU subgroups, but none of these differences reached statistical significance. In the same way, we found very similar proportions of CCR5 genotypes among infected and uninfected IDUs. These observations speak in favor of future large studies and efforts to integrate data from different individual studies involving similar populations. Such large studies are urgently needed in Brazil and most developing countries. Despite this, the present report is a pioneer descriptive study of the distribution of CCR5 genotypes and HLA-B alleles in the IDU population from Rio de Janeiro, Brazil.

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References

Alvarez, V., López-Larrea, C., Coto, E., 1998. Mutational analysis of the CCR5 and CXCR4 genes (HIV-1 co-receptors) in resistance to HIV-1 infection and AIDS development among intravenous drug users. Hum. Genet. 102, 483–486.
Alves, C., Meyer, I., Vieira, N., Toralles, M.B.P., LeMaire, D., 2006. Distribution and frequency of HLA alleles and haplotypes in Brazilians with type 1 diabetes mellitus. Arq. Bras. Endocrinol. Metab. 50, 436–444.
Carrington, M., O'Brien, S.J., 2003. The influence of HLA genotype on AIDS. Annu. Rev. Med. 54, 535–551.
de Sorrentino, A.H., Marinic, K., Motta, P., Sorrentino, A., Lopez, R., Illiovich, E., 2000. HLA Class I alleles associated with susceptibility or resistance to human immunodeficiency virus type 1 infection among a population in Chaco Province, Argentina. J. Infect. Dis. 182, 1523–1526.
Dean, A.G., Dean, J.A., Coulombier, D., Brendel, K.A., Smith, D.C., Burton, A.H., Dicker, R.C., Sullivan, K., Fagan, R.F., Amer, T.G., 1995. Epi Info, Version 6: A word Processing, Database, and Statistics Program for Epidemiology on Microcomputers. Centers for Disease Control and Prevention, Atlanta, GA, USA.
Dean, M., Carrington, M., Winkler, C., Huttley, G.A., Smith, M.W., Allikmets, R., Goedert, J.J., Buchbinder, S.P., Vittinghoff, E., Gomperts, E., Donfield, S., Vlahov, D., Kaslow, R., Saah, A., Rinaldo, C., Detels, R., O'Brien, S.J., 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia growth and development study, Multi-

center AIDS Cohort study, multicenter Hemophilia cohort study, San Francisco city Cohort, ALIVE study. Science 273, 1856–1862.
Fabio, G., Smeraldi, R.S., Gringeri, A., Marchini, M., Bonara, P., Mannucci, P.M., 1990. Susceptibility to HIV infection and AIDS in Italian haemophiliacs is HLA associated. Br. J. Haematol. 75, 531–536.
Goldberg, A.C., Chiarella, J.M., Marin, M.L.C., Rosales, C., Banic, D., Oliveira, M.A., Rodrigues, H., Viggiani, C.S., Kalil, J., 1998. Molecular typing of HLA Class II antigens in a São Paulo population. Genet. Mol. Biol. 21, 301–305.
Goulder, P.J., Watkins, D.I., 2008. Impact of MHC Class I diversity on immune control of immunodeficiency virus replication. Nat. Rev. Immunol. 8, 619–630.
Grimaldi, R., Shindo, N., Acosta, A.X., Dourado, I., Brites, C., de Melo Carvalho, O., Brito, I., Bou-Habib, D.C., Galvao-Castro, B., 2002. Prevalence of the CCR5Δ32 mutation in Brazilian populations and cell susceptibility to HIV-1 infection. Hum. Genet. 111, 102–104.
Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48, 361–372.
Hacker, M.A., Friedman, S.R., Telles, P.R., Teixeira, S.L.M., Bongertz, V., Morgado, M.G., Bastos, F.I., 2005. The role of ''long-term'' and ''new'' injectors in a declining HIV/AIDS epidemic in Rio de Janeiro, Brazil. Subst. Use Misuse 40, 99–123.
Hildebrand, W.H., Madrigal, J.A., Belich, M.P., Zemmour, J., Ward, F.E., Williams, R.C., Parham, P., 1992. Serologic cross-reactivities poorly reflect allelic relationships in the HLA-B12 and HLA-B21 groups. Dominant epitopes of the alpha 2 helix. Immunology 149, 3563–3568.
Huang, Y., Paxton, W.A., Wolinsky, S.M., Neumann, A.U., Zhang, L., He, T., Kang, S., Ceradini, D., Jin, Z., Yazdanbakhsh, K., Kunstman, K., Erickson, D., Dragon, E., Landau, N.R., Phair, J., Ho, D.D., Koup, R.A., 1996. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat. Med. 2, 1240–1243.
IBGE. 2000. Brazilian Institute of Geography and Statistics. Agregated Database. Automated Recovery IBGE System–SIDRA. Demographic Census. (Available at: http://www.sidra.ibge.gov.br. Accessed: January, 24th, 2007).
Kiepiela, P., Leslie, A.J., Honeyborne, I., Ramduth, D., Thobakgale, C., Chetty, S., Rathnavalu, P., Moore, C., Pfafferott, K.J., Hilton, L., Zimbwa, P., Moore, S., Allen, T., Brander, C., Addo, M.M., Altfeld, M., James, I., Mallal, S., Bunce, M., Barber, L.D., Szinger, J., Day, C., Klenerman, P., Mullins, J., Korber, B., Coovadia, H.M., Walker, B.D., Goulder, P.J., 2004. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature 432, 769–774.
Kitts, A., Feolo, M., Helmberg, W., 2003. The Major Histocompatibility Complex Database, dbMHC. In: McEntyre, J., Ostell, J. (Eds.), The NCBI Handbook (Internet). National Library of Medicine (US) National Centre for Biotechnology Information, p. 29.
Lancaster, A., Nelson, M.P., Single, R.M., Meyer, D., Thomson, G., 2003. PyPop: a software framework for population genomics: analyzing large-scale multilocus genotype data. In: Altman, R.B., et, al. (Eds.), Pacific Symposium on Biocomputing 8. World Scientific, Singapore, pp. 514–525.
Liu, R., Paxton, W.A., Choe, S., Ceradini, D., Martin, S.R., Horuk, R., MacDonald, M.E., Stuhlmann, H., Koup, R.A., Landau, N.R., 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 86, 367–377.
Louzada-Junior, P., Smith, A.G., Hansen, J.A., Donadi, E.A., 2001. HLA-DRB1 and–DQB1 alleles in the Brazilian population of the northeastern region of the state of São Paulo. Tissue Antigens 57, 158–162.
Lucotte, G., 1997. Frequencies of the CC chemokine receptor 5Δ32 allele in various populations of defined racial background. Biomed. Pharmacother. 51, 469–473.
MacDonald, K.S., Fowke, K.R., Kimani, J., Dunand, V.A., Nagelkerke, N.J., Ball, T.B., Oyugi, J., Njagi, E., Gaur, L.K., Brunham, R.C., Wade, J., Luscher, M.A., Krausa, P., Rowland-Jones, S., Ngugi, E., Bwayo, J.J., Plummer, F.A., 2000. Influence of HLA supertypes on susceptibility and resistance to human immunodeficiency virus type 1 infection. J. Infect. Dis. 181, 1581–1589.
Marmor, M., Sheppard, H.W., Donnell, D., Bozeman, S., Celum, C., Buchbinder, S., Koblin, B., Seage 3rd., G.R., HIV Network for Prevention Trials Vaccine Preparedness Protocol Team, 2001. Homozygous and heterozygous CCR5Δ32 genotypes are associated with resistance to HIV infection. J. Acquir. Immune Defic. Syndr. 27, 472–481.
Martinson, J.J., Chapman, N.H., Rees, D.C., Liu, Y.T., Clegg, J.B., 1997. Global distribution of the CCR5 gene 32-basepair deletion. Nat. Genet. 16, 100–103.
Monte, S.J., Moita Neto, J.M., Rampim, G.F., Shulzhenko, N., Morgun, A., Gerbase-DeLima, M., Grupo, HLA-UFPI., 2004. HLA polymorphism in a racially admixed sample of the population of Teresina, Piaui. Rev. Assoc. Med. Bras. 50, 422–426 (Article in Portuguese).
Moraes, M.E., Fernandez-Viña, M., Salatiel, I., Tsai, S., Moraes, J.R., Stastny, P., 1993. HLA Class II DNA typing in two Brazilian populations. Tissue Antigens 41, 238–242.
Moraes, M.E., Romero, M., Salomão, I., Correa, M., Gouvêa, N., Romero, M., Hue, M.I., Fernandez-Viña, M.A., Moraes, J.R., 2004. Strong linkage disequilibrium between HLA-B*3913 and DRB1*0807 in Brazilians. Transplant. Proc. 36, 823–824.
Nigam, P., Dellalibera, E., Mauricio-da-Silva, L., Donadi, E.A., Silva, R.S., 2004. Polymorphism of HLA Class I genes in the Brazilian population from the Northeastern State of Pernambuco corroborates anthropological evidence of its origin. Tissue Antigens 64, 204–209.
O'Brien, S.J., Nelson, G.W., 2004. Human genes that limit AIDS. Nat. Genet. 36, 565–574.
Oliveira, M.L., Yoshida, C.F., Telles, P.R., Hacker, M.A., Oliveira, S.A., Miguel, J.C., do, Ó.K.M, Bastos, F.I., 2009. Trends in HCV prevalence, risk factors and distribution of viral genotypes in injecting drug users: findings from two cross-sectional studies. Epidemiol. Infect. 15, 1–10.
Robinson, J., Waller, M.J., Parham, P., de Groot, N., Bontrop, R., Kennedy, L.J., Stoehr, P., Marsh, S.G.E., 2003. IMGT/HLA and IMGT/MHC: sequence databases for the study of the Major Histocompatibility Complex. Nucleic Acids Res. 31, 311–314.
Rowland-Jones, S.L., Nixon, D.F., Aldhous, M.C., Gotch, F., Ariyoshi, K., Hallam, N., Kroll, J.S., Froebel, K., McMichael, A., 1993. HIV-specific cytotoxic T-cell activity in an HIV-exposed but uninfected infant. Lancet 341, 860–861.
Rowland-Jones, S., Sutton, J., Ariyoshi, K., Dong, T., Gotch, F., McAdam, S., Whitby, D., Sabally, S., Gallimore, A., Corrah, T., Takiguchi, M., Schultz, T., McMichael, M., Whittle, H., 1995. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nat. Med. 1, 59–64.
Royce, R.A., Sena, A., Cates Jr., W., Cohen, M.S., 1997. Sexual transmission of HIV. N. Engl. J. Med. 336, 1072–1078.
Ruiz, T.M., da Costa, S.M., Ribas, F., Luz, P.R., Lima, S.S., da Graça Bicalho, M., 2005. Human leukocyte antigen allelic groups and haplotypes in a Brazilian sample of volunteer donors for bone marrow transplant in Curitiba, Paraná. Brazil. Transplant. Proc. 37, 2293–2296.
Samson, M., Libert, F., Doranz, B.J., Rucker, J., Liesnard, C., Farber, C.M., Saragosti, S., Lapoumeroulie, C., Cognaux, J., Forceille, C., Muyldermans, G., Verhofstede, C., Burtonboy, G., Georges, M., Imai, T., Rana, S., Yi, Y., Smyth, R.J., Collman, R.G., Doms, R.W., Vassart, G., Parmentier, M., 1996. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 382, 722–725.
Sullivan, A.D., Wigginton, J., Kirschner, D., 2001. The co-receptor mutation CCR5Δ32 influences the dynamics of HIV epidemics and is selected for by HIV. PNAS 98, 10214–10219.
Teixeira, S.L.M., Bastos, F.I., Telles, P.R., Hacker, M.A., Brigido, L.F., Oliveira, C.A.F., Bongertz, V., Morgado, M.G., 2004. HIV-1 infection among injection and ex-injection drug users from Rio de Janeiro, Brazil: prevalence, estimated incidence and genetic diversity. J. Clin. Virol. 31, 221–226.
Telenti, A., 2005. Adaptation, co-evolution, and human susceptibility to HIV-1 infection. Infect. Genet. Evol. 5, 327–334.
Theodorou, I., Capoulade, C., Combadiere, C., Debre, P., 2003. Genetic control of HIV disease. Trends Microbiol. 11, 392–397.
Trachtenberg, E.A., Erlich, H.A., 2001. A Review of the Role of the Human Leukocyte Antigen (HLA) System as a Host Immunogenetic Factor Influencing HIV Transmission and Progression to AIDS. In: Korber, B.T.K., Brander, C., Haynes, B.F., Koup, R., Kuiken, C., Moore, J.P., Walker, B.D., Watkins, D. (Eds.), HIV Molecular Immunology. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, LA-UR 02-2877, p. I-43-60.
Vanderborght, P.R., Pacheco, A.G., Moraes, M.E., Antoni, G., Romero, M., Verville, A., Thai, V.H., Huong, N.T., Ba, N.N., Schurr, E., Sarno, E.N., Moraes, M.O., 2007. HLA-DRB1*04 and DRB1*10 are associated with resistance and susceptibility, respectively, in Brazilian and Vietnamese leprosy patients. Genes Immun. 8, 320–324.
Vargas, A.E., Marrero, A.R., Salzano, F.M., Bortolini, M.C., Chies, J.A.B., 2006. Frequency of CCR5Δ32 in Brazilian populations. Braz. J. Med. Biol. Res. 39, 321–325.
Williams, F., Meenagh, A., Darke, C., Acosta, A., Daar, A.S., Gorodezky, C., Hammond, M., Nascimento, E., Middleton, D., 2001. Analysis of the distribution of HLA-B alleles in populations from five continents. Hum. Immunol. 62, 645–650.